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A RETROSPECTIVE SEROLOGICAL STUDY OF JAPANESE WHO CONTRACTED DENGUE FEVER IN THAILAND

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SUMMARY Between September and November 1981, some members of a survey team from Japan suffered from a febrile illness diagnosed clinically as dengue fever during their stay in a village in Khon-Kaen Province, in the north-eastern part of Thailand. The morbidity rate in the team was as high as 69% (11/16). Blood samples were taken from 12 of the 16 members of the team in February, 1982 in Japan and the serum specimens were examined for antibodies to dengue (DEN), Japanese encephalitis (JE) and yellow fever (YF) viruses respectively. The results of the tests indicated that all 8 members who had had symptoms had been infected with DEN type 1 virus. No case of inapparent infection with dengue viruses was found. Of these 8 persons, seven had had neutralizing (N) antibody to JE virus before infection, but their clinical manifestations had been similar to those of an individual without N antibody to JE virus and were typical symptoms of dengue fever, such as leukopenia and " saddle-back " fever, without hemorrhagic manifestations, as seen from platelet counts and hematocrit values.

INTRODUCTION

Recently, the frequency of visits of Japanese people to Southeast Asian countries for academic and commercial reasons has been increasing. The staff of the Center for Southeast Asian Studies, Kyoto University, requested us to make serological examinations on a dengue fever-like illness that had occurred among members of a survey team to Khon-Kaen. They also asked us about their possible secondary dengue infection in the future. We have studied the epidemiology of arbovirus infections in Thailand (Fukunaga et al., 1974a; Fukunaga et al., 1974b; Okuno et al., 1980; Fukunaga et al., 1980). Thus we were interested in serological examinations on the members of this team, because, if serologically confirmed, they would be the first cases of dengue infection in a group of Japanese people in Southeast Asia and because these examinations would give some information on the effect of pre-existing antibody to JE virus on primary dengue infection. It seemed important to determine the effect of pre-existing antibody to JE virus, which like dengue viruses is a flavivirus, on primary dengue infection, since JE virus like dengue viruses is endemic

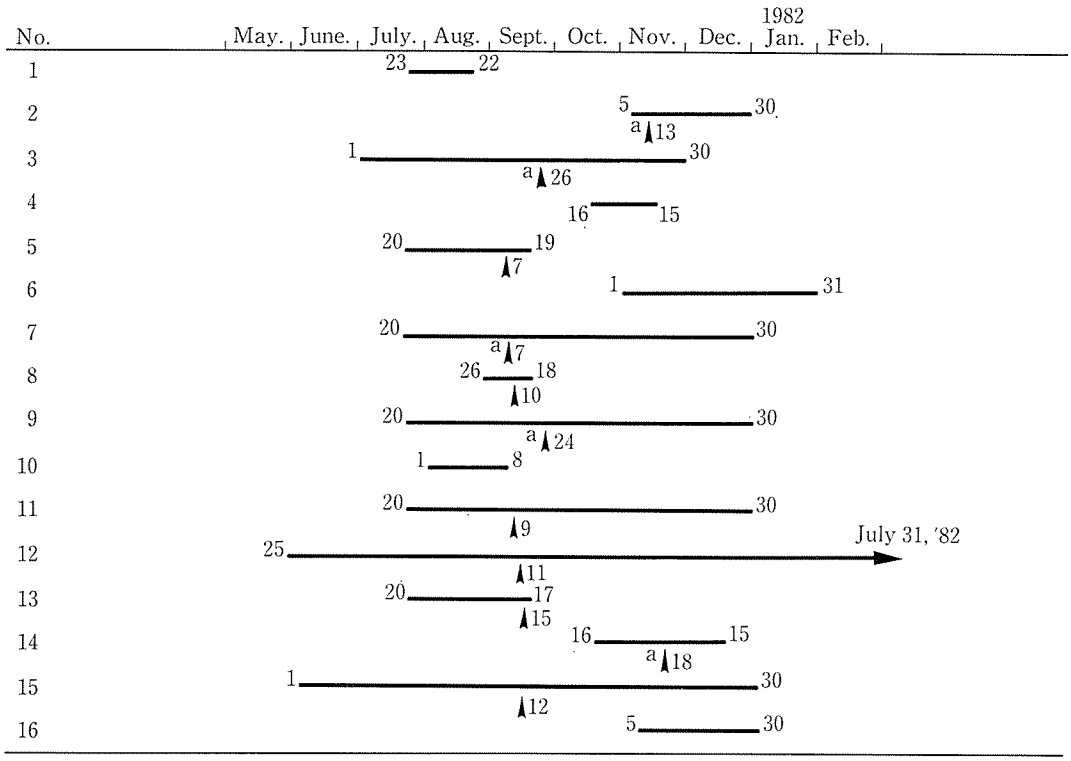

FIGURE 1. Periods of stay of members of the survey team in Khon-Kaen and dates when dengue fever-like symptoms developed.

[a] Hospitalized.

Arrowhead: onset of illness.

in the northern part of Thailand and large scale vaccination against JE virus is considered to be an urgent requirement.

The objectives of this study were as follows: (1) To confirm that the members of the team had been infected with dengue virus in Khon-Kaen. (2) To identify the serotype of this dengue virus. (3) To confirm that the infections were primary dengue infections. (4) To estimate the effect of pre-existing antibody to JE virus on primary dengue infection.

This paper reports the results of serological tests and clinical examinations of these subjects in a hospital in Khon-Kaen, in relation with the objectives mentioned above.

MATERIALS AND METHODS

1. *Hemagglutination inhibition (HI) tests*

The antigens used in HI tests were prepared by the sucrose-acetone extration method from the brain of suckling mice infected with the prototype dengue type 1 (DEN-1), Hawaiian strain, type 2 (DEN-2), New Guinea B strain, type 3 (DEN-3), H-87 strain, type 4 (DEN-4), H-241 strain and the Nakayama strain of JE virus (JE (Nak)), which is the vaccine strain used in Japan. HI tests were performed by the method of Clarke and Casals (1958) as modified for a microsystem (Sever, 1962), using 8 units of hemagglutinating antigens.

2. *Neutralization tests*

The neutralizing (N) antibody tiers of the serum specimens to DEN-1–4, JE (Nak) viruses and the

TABLE 1. *Age, sex and answers on vaccine history, suspected dengue infection and hospitalization of subjects*

No.	Age	Sex	Vaccine (JE and YF)	Dengue-like infection	Hospitalized
1	52	M	JE: 1965 (?) YF: 1965	Yes (Korat, 1961)	Yes
2	39	M	JE: 2 shots, Oct., 1981 YF: No	Yes	Yes
3	42	M	JE: Probably no YF: No	Yes	Yes
4	48	M	JE: Probably no YF: No	No	—
5	50	M	JE: 1948–50 YF: No	Yes	No
6	36	M	JE: 2–3 shots, "When"?	No	—
7	36	M	JE: No memory YF: No	Yes	Yes
9	28	M	JE: 2–3 shots, 1970 YF: 1980	Yes	Yes
10	55	M	JE: No in previous 5 year YF: No	No	—
11	34	M	JE: No memory YF: No	Yes	No
13	33	M	JE: 2–3 shots, 1972 YF: No	Yes	No
15	30	M	JE: No memory YF: 1972	Yes	No

17D strain of yellow fever virus (YF (17D)) were measured by focus reduction methods applying the peroxidase-anti-peroxidase (PAP) staining technique (Okuno et al., 1978). Titers are expressed as 50% focus reduction rates (FR_{50}). For the tests, BHK21 cells cultured on Lab-Tek 8 chamber slides (Miles, Ill., U.S.A.) were used. Serum specimens were heat-inactivated at 56 C for 30 min before the test.

RESULTS

1. *Periods of stay in Khon-Kaen of participants in the survey and dates when their dengue fever-like symptoms developed*

A total of 16 social scientists participated in the settled surveillance of a village in Khon-Kaen Province. Their periods of stay at the survey site varied for one month to more than 14 months. As shown in Fig. 1, 11 of the 16 participants developed dengue fever-like symptoms (morbidity rate: 69%) and most (9/11) cases occurred in September. Five of the 11 patients were admitted in Som Memorial Hospital in Khon-Kaen for about one week.

2. *Age, sex, vaccine history, dengue fever-like infection and hospitalization of subjects from whom serum specimens were collected in Japan*

On Feb. 3, 1982, blood was taken from 12 subjects for serological tests and their histories of vaccination against Japanese encephalitis (JE) and yellow fever (YF) viruses and of dengue-like disease were recorded. These histories are summarized in Table 1. Subject No. 1 in Table 1, who had contracted a dengue fever-like illness and been hospitalized in Korat, Thailand, in 1961, had not developed any symptoms in Khon-Kaen. Most of the subjects were uncertain of their history of JE vaccination, since, if at all, they had been vaccinated more than five years previously. However, as seen in Table 1, subject No. 2 had received two shots of killed JE vaccine one month before his symptoms developed in Khon-Kaen. Regarding histories of YF vaccine, three subjects (No. 1, 9, 15) gave

TABLE 2. *Clinical manifestations*

No.	Duration of fever (day)	Maximum body temperature (C)	Rash	Other symptoms
2	7	39.7	Yes	Loss of appetite, Headache, Lumbago, Mild diarrhea
4	7	40.0	No	Malaise, Loss of appetite
5	4	39.0	Yes	Malaise, Loss of appetite, Lumbago
7	7	39.0	Unclear	Malaise, Backache and Lumbago, Loss of appetite lasting 2 weeks
9	4	39.0	No	Extreme loss of appetite
11	10	39.0	Yes	Malaise, Headache, Loss of appetite
13	5	40.0	No	Pain in muscles and joints of extremities, Lumbago, Mild diarrhea
15	7	38.5	Yes	Malaise, Loss of appetite, Headache, Retroorbital pain

definite answers, but two of them had been vaccinated a considerable time previously.

The clinical manifestations recognized are summarized in Table 2. The predominant subjective symptoms were malaise and loss of appetite, and half the patients noticed the appearance of a rash.

3. *Results of hemagglutination inhibition (HI) tests*

The results of HI tests on 12 serum specimens for DEN-1–4 and JE (Nak) antigens are shown in Table 3. Subjects who had not developed symptoms in Khon-Kaen (No. 4, 6, 10) showed no HI antibody to either type of dengue virus. On the other hand, the serum specimens from those who had developed symptoms showed broadly reacting HI antibodies, especially when they had high (1: 320 or more) HI titers to JE (Nak) antigen. The serotype of the infecting dengue virus could not be identified from these results.

TABLE 3. *Results of HI-tests*

No.	HI-antibody titer				
	DEN-1	DEN-2	DEN-3	DEN-4	JE(Nak)
1	10	<10	80	<10	20
2	320	160	160	320	640
3	80	10	20	20	80
4	<10	<10	<10	<10	<10
5	160	80	160	160	320
6	<10	<10	<10	<10	<10
7	40	10	20	10	40
9	40	10	20	20	40
10	<10	<10	<10	<10	20
11	160	80	80	80	640
13	80	<10	10	10	20
15	160	40	80	40	320

4. *Results of Neutralization (N) tests*

Table 4 shows the results of N tests on 12 serum specimens for DEN-1–4, JE (Nak) and YF (17 D) viruses. The three subjects who had had no symptoms had no detectable N antibody to either serotype of dengue virus. Thus no case of inapparent infection with dengue viruses was found in this study. All subjects who had had symptoms except No. 2 were found to have N antibody to only DEN-1 virus. This was present at rather high levels and showed clear type-specificity, indicating that the subjects had been primarily infected with DEN-1 virus in Khon-Kaen. Though subject No. 2 had low levels of antibody to DEN-2, DEN-3 and DEN-4 viruses, he had

TABLE 4. *Results of neutralization tests*

No.	Neutralizing antibody titer (FR_{50})					
	DEN-1	DEN-2	DEN-3	DEN-4	JE(Nak)	YE(17D)
1	10	310	290	<10	1450	<10
2	1250	19	22	14	2700	<10
3	390	<10	<10	<10	160	<10
4	<10	<10	<10	<10	<10	nd
5	880	<10	<10	<10	480	<10
6	<10	<10	<10	<10	600	nd
7	290	<10	<10	<10	180	nd
9	310	<10	<10	<10	110	180
10	<10	<10	<10	<10	510	nd
11	610	<10	<10	<10	500	nd
13	450	<10	<10	<10	<10	nd
15	720	<10	<10	<10	220	<10

high levels of N antibody to DEN-1 (1: 1250) and JE (1: 2700) viruses. Therefore, his low level titers to DEN-2, DEN-3 and DEN-4 viruses seemed to result from the effect of the high N antibody titer to JE (Nak) virus caused by his recent two shots of killed JE vaccine, rather than from secondary dengue infection. Actually, subject No. 2 had never experienced dengue fever-like symptoms before this occasion and usually, the convalescent phase serum of patients with secondary dengue infection shows similar N antibody titers to all 4 serotypes (Okuno et al., 1980).

The N antibody to YF (17 D) virus was detected in only one subject, No. 9, who thought that he remembered having been inoculated with live YF vaccine in 1980. Two other subjects who thought that they had received live YF vaccine in 1965 and 1972, respectively, had no detectable N antibody to YF virus.

5. *Effect on primary dengue infection of pre-existing antibodies to JE and/or YF virus*

Among 8 subjects who contracted dengue fever-like symptoms in Khon-Kaen, only one (No. 13) had no N antibody to JE virus before the primary dengue infection occurred. Therefore, the infection with DEN-1 virus in Khon-Kaen was a primary infection with flavivirus in subject No. 13, but a secondary infection with a flavivirus in the other 7 subjects, because they had pre-existing antibodies to JE and YF viruses. It should be noted that before the DEN-1 infection occurred subject No. 2 had had a high antibody titer to JE virus, probably caused by two shots of JE vaccine, and also that subject No. 9 had had antibodies to both JE and YF viruses. As seen in Table 5, the record of clinical examinations on subject No. 2 on admission to hospital (Som Memorial Hospital in Khon-Kaen) showed that he had not developed hemorrhagic manifestation such as decrease of the platelet count or increase of the hematocrit value, but had developed leukopenia which is considered the most significant laboratory finding in patients with dengue fever (Schlesinger, 1977). Similarly, subject No. 9, who had had antibodies to

TABLE 5. *Clinical examinations of subject No. 2*

Date	WBC	Platelets	Hematocrit
Nov. 14	5500 /mm³	Adequate /mm³	40%
15	4000	Adequate	40
16	2900	350,000	45
17	3600	Adequate	46
18	3200	Adequate	47
19	3500	Adequate	42
20	5350	Adequate	44

JE and YF viruses, had developed leukopenia without either increase of the hematocrit value or decrease of the platelet count. The other patients admitted to hospital showed similar records of clinical examinations to those of subjects Nos. 2 and 9.

DISCUSSION

This study demonstrated that some members of a survey team from Japan had been infected with DEN-1 virus in Khon-Kaen, Thailand, and developed typical symptoms of dengue fever. The morbidity rate was as high as 69% in the team and this illness must have seriously hampered the activities of the team. Therefore, vaccines to dengue viruses must be developed even for Japanese, because although dengue viruses are not endemic in Japan, many Japanese must visit and stay in countries of southeast Asia.

Anamnestic immune responses in sequential infections with flaviviruses have been an important subject with regard to protection from flavivirus infections. Since the " hypersensitivity " theory was proposed for the pathogenesis of dengue hemorrhagic fever (DHF) by Halstead, Russell and their associates in 1960's, the role of anamnestic immune responses in sequential flavivirus infections has become a more realistic problem. Furthermore, the same workers recently added the hypothesis of " immunological enhancement of infection " theory, indicating that flavivirus group-specific antibody may have a role in enhancement of dengue virus infections (Halstead, 1981). Halstead and his colleagues mainly concentrated their studies on virus sequences within the dengue subgroup, but experimental and epidemiological observations on the role of anamnestic immune reponses in sequential infections with dengue and other flaviviruses have been reported by others (Sabin, 1952; Grossberg and Scherer, 1958: Carey et al., 1965; Wisseman et al., 1966; Bancroft et al., 1980; Okuno et al., 1982). None of the cases reported showed any predisposition to aggravated illness on subsequent infection. In this study also, seven of eight patients had had N antibody to JE virus before the DEN-1 virus infection occurred, but their symptoms were no different from those of a patient without N antibody to JE virus and were typical dengue fever symptoms without hemorrhagic manifestations, as seen by their platelet counts and hematocrit values. It should be noted that subject No. 2 had received two shots of JE vaccine one month before DEN-1 virus infection and also that subject No. 9 had had N antibodies to both JE and YF viruses before infection with DEN-1. However, it cannot be concluded that pre-existing antibodies to JE and/or YF viruses, which belong to a different subgroup of DEN viruses (DeMadrid and Porterfield, 1974), do not modify hemorrhagic symptoms in primary dengue infection, because the frequency of DHF or the dengue shock syndrome (DSS) is estimated to be 5.5—36 per 1000 cases of secondary dengue infection (Halstead, 1980).

When volunteers, previously immunized with YF vaccine, were given live DEN-2 vaccine, they showed N antibody responses not only to the homologous DEN-2 virus but also to other serotypes of dengue viruses (Bancroft et al., 1980). Our obsevations on a case of laboratory dengue infection are consistent with this finding (Okuno et al., 1982): One of the authors of this paper, who had been immunized to JE virus, suffered primary dengue infection with a strain of DEN-4 virus, which was demonstrated by detection of IgM antibody specific to DEN-4 virus. However, his convalescent phase sera showed almost the same levels of N antibodies to all 4 serotypes of dengue viruses but no significant increase of N antibody to JE virus. Thus sequential vaccinations, such as with YF vaccine and then live DEN-2 vaccine, or with JE vaccine and then live DEN-2 vaccine, seem to be advantageous because a single serotype vaccine of dengue viruses can produce N antibodies to all 4 serotypes of dengue viruses. However, we found that the durations of persistence of N

antibodies to heterologous serotypes were shorter than that to the homologous serotype. Consistent with previous findings, in this study, subject No. 2, who received JE vaccine before infection, showed N antibody responses to not only the serotype of infecting virus (DEN-1) but also three other serotypes though at lower titers than that to the homologous serotype. In subject No. 9, who was immune to YF virus before DEN-1 virus infection, no N antibodies to heterologous serotypes were detected, possibly because his blood sample was taken longer after infection than that of subject No. 2.

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